(12) UK Patent Application (19) GB (11) 2 102 811 A

(21) Application No 8219500
(22) Date of filing 6 Jul 1982
(30) Priority data
(31) 3337/81
(32) 28 Jul 1981
(33) Austria (AT)
(43) Application published
9 Feb 1983
(51) INT CL³
C07G 7/00
A61K 37/02
(52) Domestic classification
C3H K1 K2
A5B 170 180 215 216 21Y
320 32Y 38Y 39X J
U1S 1311 1320 C3H
(56) Documents cited
None
(58) Field of search
C3H
A5B
(71) Applicants
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Chemischmedizinische
Produkte,
(At
72
12
At
(72) In
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(54) A tissue adhesive and a method of producing the same

(57) A tissue adhesive based on human or animal proteins contains factor XIII, fibrinogen and an antibiotic. In order to achieve a high straining capacity of the adherences and a retarded antimicrobial efficacy, the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500. The antibiotic is chosen among amino-glucosides, beta-lactams, polypeptides or tetracyclines. In a method of producing the tissue adhesive the concentration ratio of factor XIII to fibrinogen is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII. The antibiotic is added either before deepfreezing or lyophilization of the resulting preparation, or after thawing or reconstitution of the same.

ERRATUM

SPECIFICATION NO. 2102811 A

Front page, Heading (71) Applicants *for* Immuno AG fur *read* Immuno Aktiengesellschaft für

THE PATENT OFFICE
*27 May 1983*

Bas 251492.

GB 2 102 811 A

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(54) **A tissue adhesive and a method of producing the same**

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SPECIFICATION

**Improvements in or relating to e tissue adhesive and a method of producing the same**

Tha invention relates to a tissue adhesive based on human or animal proteins, contalning factor XIII, fibrinogen and an antibiotic, as wall as to a method of producing the same.

From British Patent application No. 8003775 and British patent application No. 8003776 methods are already known for producing a tissue adhesive containing fibrinogen and factor XIII, in which certain concentration ratios of factor XIII to fibrinogen and, if dasired, albumin are edjusted, and the preparations are deepfrozen or lyophilized. These preparations besically exhibited satisfactory properties, i.e. a high straining capacity of the adherences and a good ebsorbability; however, it is desirable to improve these preparations with a view to an antimicrobial efficacy.

To be sura, it has already been proposed in U.S. patant No. 2,533,004 as well as by Fellinger et al. in tha journal "Der Tuberkulosearzt" (6/11,1952) to add antibiotics to fibrinogen solutions and to use them as wound adhesives, yet these solutions to be prepared directly at the baside do not give the fibrin clots formed therefrom a sufficient durability and straining capacity.

Furthermore, it is known from the work by Bösch at al., Archiv für orthopädische und Unfall-Chirurgia, Vol. 90 (1977), pages 63 to 75, to apply a fibrin adhesive system for filling bone defects in connection with bone transplants, with the fibrin forming at the chosen site immediataly at the bone cavity by the addition of thrombin to a fibrinogen solution. As required, commonly available combination preparations of pulverized neomycin were added.

Finally, it was proposed according to the PCT application No. 80/00083 to prapere a fibrinogen antibiotic gel, wherein a mixture of cryoprecipitate with tobramycin and gentamicin as antibiotics to be prepared at the badside is used.

According to experiments carried out by Applicant it was found that the described and known tissue adhesives that contain fibrinogen, factor XIII and antibiotic do not possess the desired combination of properties, i.e. a high straining capacity of the adherences and an antimicrobial efficacy, but that an advarse interaction between the antibiotics and factor XIII takes place, whlch rasults in a strong decrease of the cross-linking ability of fibrinogen and a negative effect on the coagulability. Consequently, the adhesive exhibits a poorer rigidity and adhering capacity on the wound and tissue surfaces.

A further disadvantage of tha known preperations resides in that the release of the antibiotic to tha tissue takes place too quickly so that the retardation of the antibiotic does not suffice to be effective over a longer period of tima and to achieve a high active substance release.

The invention aims at avoiding these disedvantages and difficulties and has as its object to provide a tissue adhesive of human or animal origin that meets the above-mentioned combination of properties end guarantees an improved efficacy of the antibiotic.

The set object is achieved with a tissue adhesive of the initially defined kind in that tha ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500 and that an antibiotic selected from the group consistlng of aminoglucosides, betalactams, polypeptides and tetracyclines is contained therein.

Advantagaously, factor XIII is containad in a deepfrozen tissue adhasive in an amount of at least 40 units/ml.

According to another embodiment at leest 33 % of fibrinogen is contained in a lyophilized tissue adhesive, factor XIII being present in an amount of at leest 170 units/g of lyophilisate.

Suitably, a plasmin Inhibitor or plasminogen-activator inhibitor salected from the group consisting of aprotinin, $\alpha_2$-antiplasmin, $\alpha_2$-macroglobulin, $\alpha_1$-antitrypsin, ε-aminocaproic acid and tranexamlc acid is additionally contained.

Advantageously, the tissue adhasive is a two-component preparation, factor XIII, fibrinogen and the plasmin inhibitor or plasminogen-activator inhibitor being contained in the first component and the antibiotic, thrombin and bivalent calclum baing contained in the second component.

Preferably, the antiblotic is contained in the form of a hardly solubla derivative. A variant of this embodiment consists in that, in addition to the hardly soluble derivative, also an easily soluble one is used, if desired distributed in the two components of tha tissue adhesive. This embodiment has the advantage that the easily soluble derivative is released quickly, thus ensuring a high initial efficacy, whereas the hardly soluble derivative causas a lasting efficacy.

Moreover, the invention comprises a method of producing the tissue adhesive with a modification consisting in that a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/g of fibrinogen, of at least 500 is adjusted in a fibrinogen-containing blood plasma fraction by tha addition of factor XIII, whereupon the antibiotic is addad and the preparation is deepfrozen or lyophilized.

According to anothar modification a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/g of fibrinogan, of at least 500 is adjusted in a fibrinogen-containing blood plasma fraction by the addition of factor XIII, whereupon the preparation is deepfrozen or lyophilized and, after thawing or reconstitution, is combined with an antibiotic-containing solution. With this embodiment the antibiotic may be added after thawing or to the reconstituted solution. However, with these embodiments it has to be taken care that the concentration of factor XIII does not fall below a minimum concentration; it is to be above 40

units/ml.

According to preferred embodiment in which the fibrinogen-containing blood plasma fraction is washed with a buffer solution, the washing procedure is carried out until a factor XIII concentration of 200 units of factor XIII/g of fibrinogen is reached, whereupon factor XIII is supplied in an amount of at least 300 units/g of fibrinogen in the form of a concentrate of lyophilisate.

The tissue adhesive according to the invention and the method of producing the same will be explained in more detail by way of the following exemples.

*Example 1:*

Cryoprecipitate (100 g) was gained from frozen fresh human plasma by heating to 2° C, separated by centrifugation and washed twice in a buffer solution containing $Na_3$ citrate, NaCl, glycine, glucose, aprotinin and heparin at a pH of 6.5, and the separated precipitate was dissolved in a glycine-containing buffer solution (255 ml) at a pH of 7.9. It was found that a ratio of factor XIII to fibrinogen of 226 units of factor XIII/g of fibrinogen was contained in this solution. To adjust the ratio desired according to the invention, of more than 500 U/g of fibrinogen, a pulverized factor XIII preparation with 9,000 units was added to the solution, the concentration ratio of the solution thus having been increased to 826 units of fector XIII/g of fibrinogen. This solution was sterile-filtered; then 1.7g of gentamicin were added under sterile conditions, the mixture was filled into final containers (2.5 ml), deepfrozen and lyophilized.

*Example 2:*

The preparation of the tissue adhesive basis from cryoprecipitate was effected in the same manner as in Example 1, with the difference that the precipitation was liquefied by heating to 37° C after a single washing, and 13,600 units of pulverized facter XIII were added. A ratio of factor XIII to fibrinogen of 967 factor XIII units/g of fibrinogen was obtained.

5.67 g 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid were added to the solution as an antibiotic. The suspension thus obtained was filled into final containers (1 ml) and deepfrozen. The filled-in preparation has a content of factor XIII amounting to 87 U/ml.

The application of the tissue adhesives prepared according to Examples 1 and 2 advantageously is realized by mixing the thawed or reconstituted mixture with thrombin and calcium chloride and applying it onto the tissue to be connected. It is also possible to apply the two components separately onto the tissue to be connected or filled.

*Example 3:*

The method according to Example 1 was repeated except for adding the antibiotic. The washed precipitate, after dissolving in a buffer solution, was sterile-filtered, filled into final containers (2,5 ml), deepfrozen and lyophilized, the first component of the tissue adhesive according to the invention thus having been made storable. The second component was prepared prior to application from a solution of thrombin and calcium chloride by adding 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid (30 mg/ml).

*Example 4:*

The procedure according to Example 2 was repeated, wherein gentamicin (1.89 g) was added efter dissolving the cryoprecipitate, the solution was filled into final containers (1 ml) and deepfrozen. Thus, the first component of the adhesive according to the invention is present in a storable form. The second component containing 30 mg of 7-[(thienyl)-2(2)-acetamido]-cephalosporanic acid per ml of a calcium chloride thrombin solution was prepared prior to application.

Instead of the eprotinin added in accordance with Examples 1 to 4, one or more of the following compounds may be used as plasmin inhibitor or plasminogen-activator inhibitor: $\alpha_2$-antiplasmin, $\alpha_2$-macroglobulin, $\alpha_1$-antitrypsin, $\varepsilon$-aminocaproic acid and tranexamic acid.

The tissue adhesives prepared according to the invention are generally applicable for the seamless connection of human of animal tissue or organ parts, to dress wounds end stop bleedings, their antimicrobial efficacy being substantially improved.

The improved adhesive properties with an equally improved antimicrobial efficacy of the tissue adhesives according to the invention can be taken from the following comparative examples summarized in a table; the degree of crosslinking of tissue adhesives according to the invention with an increased factor XIII/fibrinogen ratio was compared to the crosslinking degree of known tissue adhesives without an increased factor XIII/fibrinogen ratio, using different antibiotics for each case. The $\alpha$-crosslinking degree has been determined according to the sodiumlaurylsulfate (SDS) polyacrylamide gel electrophoresis method, which is carried out in a manner that, after having mixed the tissue adhesive with an equal volume of a solution containing 40 µMol $CaCl_2$ and 15 NIH units U.S. National Institute of Health units) of thrombin per ml, the mixture is incubated at 37° C. The $\alpha$-crosslinking degree is determined after stopping the reaction and reductive cleavage of the disulfide bridges contained in the proteins, by the addition of a mixture of urea, sodiumdodecylsulfate and $\beta$-mercaptoethanol by means of gel electrophoresis.

In a further part of the table the clot rigidity of a tissue adhesive according to the invention was compared to a known one in a thrombelastograph, with gentamicin having been added as antibiotic.

Finally, the table includes comparative values of the tearing resistance of a tissue adhesive according to

the invention and of a known one, with gentamicin being used as an antibiotic.

Fibrin α-crosslinking (at 37°C after 60 min.)

| | Addition of antibiotic | Tissue edhesive of invention with increased factor XIII content > 500 U/g fibrinogen | Tissue edhesive without increased factor XIII content |
|---|---|---|---|
| | Gentamicin | 70 % | 30 % |
| | Neomycln | 41 % | 21 % |
| | Fosfomycin | 47 % | 24 % |
| | Axlocillin | 66 % | 42 % |
| | Doxycyclin | 65 % | 26 % |
| | Cefoxitin | 54 % | 44 % |

Clot rigidity in thrombelastograph
(37° C - 60 mln.) ε = elastlcity module

| | | |
|---|---|---|
| Gentamicin | 1150 | 426 |

Teari· esistance in g/cm²
(37° C - 30 min.)

| | | |
|---|---|---|
| Gentamicin | 1283 | 999 |

Finally, a comparative example was carried out with respect to the antibiotlcs release of e tissue edhesive prepared according to Example 4, 85 % of gentamicin having been released from a clot produced by this tissue adhesive already after 72 hours in en in vitro test. After 96 hours e release of gentemicin was not recognized any more, while 7-[(thienyl)-(2)-acetamido]-cephalosporanic acid was still detectable even after 8 days.

CLAIMS

1. A deepfrozen or lyophilized tissue adhesive based on human or enimal proteins which comprises factor XIII, fibrinogen and an antibiotic, wherein the ratio of factor XIII to fibrinogen, expressed in units of factor XIII per gram of fibrinogen, amounts to at least 500, and said antibiotic is selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines.

2. A deepfrozen tissue adhesive as set forth in claim 1, wherein factor XIII is contained in an amount of at least 40 units/ml.

3. A lyophilized tissue adhesive as set forth in claim 1, wherein at least 33 % by weight of fibrinogen is contained, factor XIII being present in an amount of at least 170 units/gram of lyophilisate.

4. A tissue adhesive es set forth in claim 1, further comprising e plasmin inhibitor or plasmlnogen-activator inhibitor selected from the group consisting of aprotinin, $\alpha_2$-antiplasmin, $\alpha_2$-macroglobulin, $\alpha_1$-antitrypsin, ε-aminocaproic acid and tranexamic acid.

5. A tissue adhesive as set forth in claim 1, comprising a first component and a second component, said first component containing factor XIII and fibrinogen and said second component contalnlng said antibiotic, thrombin and bivalent calcium.

6. A tissue adhesive as set forth in claim 4, comprising a first component end e second component, said first component containing factor XIII, fibrinogen and said plasmin inhibitor or plasminogen-activator inhibitor and said second component containing said antibiotic, thrombin and bivalent calcium.

7. A tissue adhesive as set forth in claim 1, wherein said antibiotic is present in the form of a hardly soluble derivative.

8. A method of producing a tissue adhesive based on human or animal proteins and comprising factor XIII, fibrinogen, e plesmin inhibitor or plasminogen-activator inhibitor, and an entibiotic selected from the group consisting of aminoglucosides, betalactams, polypeptides and tetracyclines, which method comprises the steps of adjusting in e fibrinogen-containing blood plasma fraction a concentration ratio of factor XIII to fibrinogen, expressed in units of factor XIII/gram of fibrinogen, of at least 500 by edding factor XIII, adding said antibiotic, and deepfreezing or lyophilizing the resulting preparetion.

9. A method of producing a tissue adhesive based on human or animal proteins and comprising factor XIII, fibrinogen, a plasmin inhibitor or plasminogen-activator inhibitor, and an antibiotic selected from the group consisting of aminoglucosides, betalectams, polypeptides and tetracyclines, which method comprises the steps of

adjusting in a fibrinogen-containing blood plasma fraction a concentratlon ratio of factor XIII to fibrinogen, expressed in units of factor XIII/gram of fibrinogen, of at least 500 by adding factor XIII,

deepfreezing or lyophilizing the resulting preparation,

thawing or reconstituting the preparation, and

combining the preparation with a solution containing said antibiotic.

10. A method as set forth in claim 8 or 9, further comprising washing said fibrinogen-containing blood plasma fraction with a buffer solution in a washing procedure, and wherein said washing procedure is carried out until a factor XIII concentration of 200 units of factor XIII/gram of fibrinogen is reached, whereupon factor XIII is added in an amount of at least 300 units/gram of fibrinogen in the form of a concentrate or lyophilisate.

11. A tissue adhesive substantially as hereinbefore described with reference to the accompanying examples.

12. A method substantially as hereinbefore described with reference to the accompanying examples.

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1983.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.